

## Coelomic Cavities May Function as a Vascular System in Amphioxus Larvae

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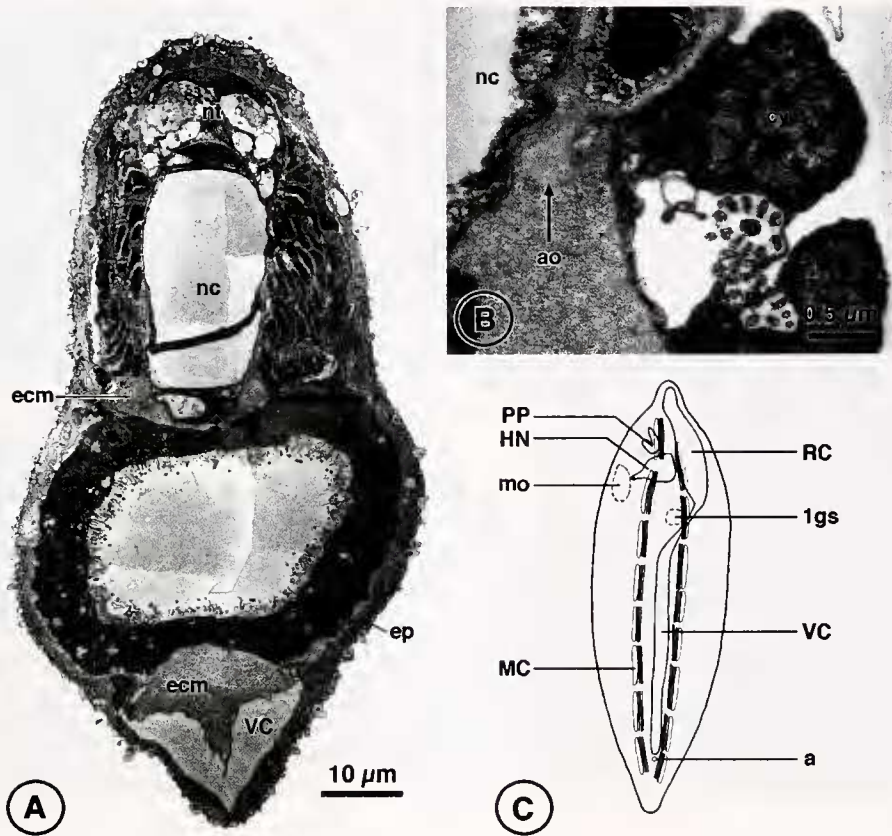
At the level of light microscopy, *Branchiostoma lanceolatum* may be the best studied species of the *Cephalochordata*—the probable sister taxon of the *Craniates* (1, 2, 3, 4). The blood vascular system of adult lancelets was studied by Rähr (5), and the detailed anatomical similarities of the major vessels prompted him to propose their homology with the vascular anatomy of *Craniates*. He also showed that, in contrast to those of the *Craniates*, most of the vessels in *B. lanceolatum* are not lined by an endothelium (6). In addition, there are rarely any hemocytes in the blood of *Cephalochordates*. Nevertheless, certain parts of the circulatory system—endostylar artery, bulbilli, glomus, hepatic portal vein, hepatic vein, sinus venosus, subintestinal vein, and others—are reported to be contractile. Ruppert (7) proposed that the myoepithelial lining of adjacent coelomic cavities may provide the driving force for such contractions. On the basis of an extensive study by transmission electron microscopy as well as light microscopical observations of living larvae, I propose that Ruppert's hypothesis also holds true for larval stages. Moreover, the observations reported here suggest that the coelomic canal system is the functional circulatory system at a stage when the blood vessels are rudimentary.

When amphioxus larvae begin to feed in the plankton (8), the extracellular matrices (ecm) of certain areas of the body are widely expanded (Fig. 1A; methods: (20)). This is particularly evident in those areas where, in adult specimens, major blood vessels will be situated. Fully developed blood vessels in adults are typically enlarged, fluid-filled spaces within the extracellular matrix (6). In

one observation, a rudimentary blood vessel could be demonstrated by electron microscopy in the expanded ecm space below the notochord of a larva with one primary gill slit (Fig. 1B). This vessel, which corresponds to the left anterior aorta (9) in the juvenile, is associated with Hatschek's nephridium, the first excretory organ to appear in the ontogeny of amphioxus. Indeed, the functioning of the nephridium may depend upon the presence of this blood vessel (10).

In living larval stages, the first observable, contractile, longitudinal vessel-like structure is situated in the ventral midline behind the first primary gill slit (labeled *vc* in Figs. 1C and 2A). This ventral structure was termed "Blutgefäß" (11), "blood vessel" (12), "artère" (13), or "longitudinal vessel which will become the endostylar and sub-intestinal vessels of the adult" (14). Peristaltic waves of contraction pass, within about 3 seconds, from the posterior end of the vessel, rostrally over its entire length of about 400  $\mu\text{m}$  (Fig. 2A). Single waves are separated from each other by a period of about 5–6 seconds. Light microscopy reveals small particles (about 0.5–1  $\mu\text{m}$ ) floating rostrally in the lumen of this vessel-like structure (Fig. 2B).

Transmission electron micrographs of larvae demonstrate (Fig. 1A) that the ventral coelom, a longitudinally extensive cavity, is situated immediately below the anlage of the sub-intestinal vessel (lower *ecm*; structure labeled *vc* in Fig. 1A; see also Fig. 1C). The location of the sub-intestinal vessel in adult lancelets is in an enlarged area of extracellular matrix of the dense connective tissue type in Rähr's nomenclature (6). This ecm-area is composed of a coarse granulated material; whereas the anlage of the left anterior aorta contains a fine granular substance (Fig. 1B) that, in electron micrographs, resembles the blood of



**Figure 1.** (A & B) Transmission electron micrographs of a larva of *Branchiostoma lanceolatum* [110 h post fertilization, 18°C; methods: (20)]. (A) Cross section of the trunk region. Structures labeled *ecm* are situated in areas where major blood vessels are situated in adults. (B) Hatschek's nephridium. Cyrtopodocytes on the enlarged *ecm* where the rudiment of the left anterior aorta is formed. (C) Diagrammatic dorsal view of coelomic cavities. a, anus; ao, anlage of the left anterior aorta; cy, cyrtopodocytic cell; *ecm*, extracellular matrix; ep, epidermis; HN, Hatschek's nephridium; mo, mouth opening; MC, myocoel; nc, notochord; nt, neural tube; PP, preoral pit; RC, rostral coelom; VC, ventral coelom; 1gs, first primary gill slit.

adults (6). Thus, the anlage of the sub-intestinal blood vessel may not contain blood fluid in this early stage. The lumen of the coelomic cavity below the anlage of the sub-intestinal blood vessel appears empty in TEM (Fig. 1A). The ventral coelom is lined by a sheet of very narrow epithelial cells interspersed with myothelial cells and cells extremely rich in rough endoplasmic reticulum (Fig. 2C, D).

The transmission electron micrographs suggest that the contractile vessel-like structure in the ventral midline of larval cephalochordates is not the sub-intestinal blood vessel, but rather the ventral coelom. The occurrence of peristaltic contractions in living specimens indicates that this system is functional in early larval stages. This precocious coelomic circulatory system could fulfill several (until now speculative) functions (see also Table 1). The observation of microscopic particles in the lumen of the

ventral coelom (Fig. 2B) suggests a role in the distribution of nutrients. This possibility is strengthened by the observation that the coelomic vessel becomes functional when the yolk reserves of nearly all cells are depleted (pers. obs.), and the animals begin to feed actively. Alternatively, since certain free coelomic cells take part in the immunological response of adult cephalochordates (15), such a function could also be hypothesized for the microscopic particles observed. Regarding the developmental stage, we might further speculate that the cells of the coelothelium, equipped with extensive rough endoplasmic reticulum (Fig. 2C), could be producing growth factors. In any event, the interpretation of the contractile vessel-like structure of larval amphioxus as a blood vessel (11, 12, 13, 14) has certainly to be revised. The contractile longitudinal vessel in these stages is a ventral coelomic canal.

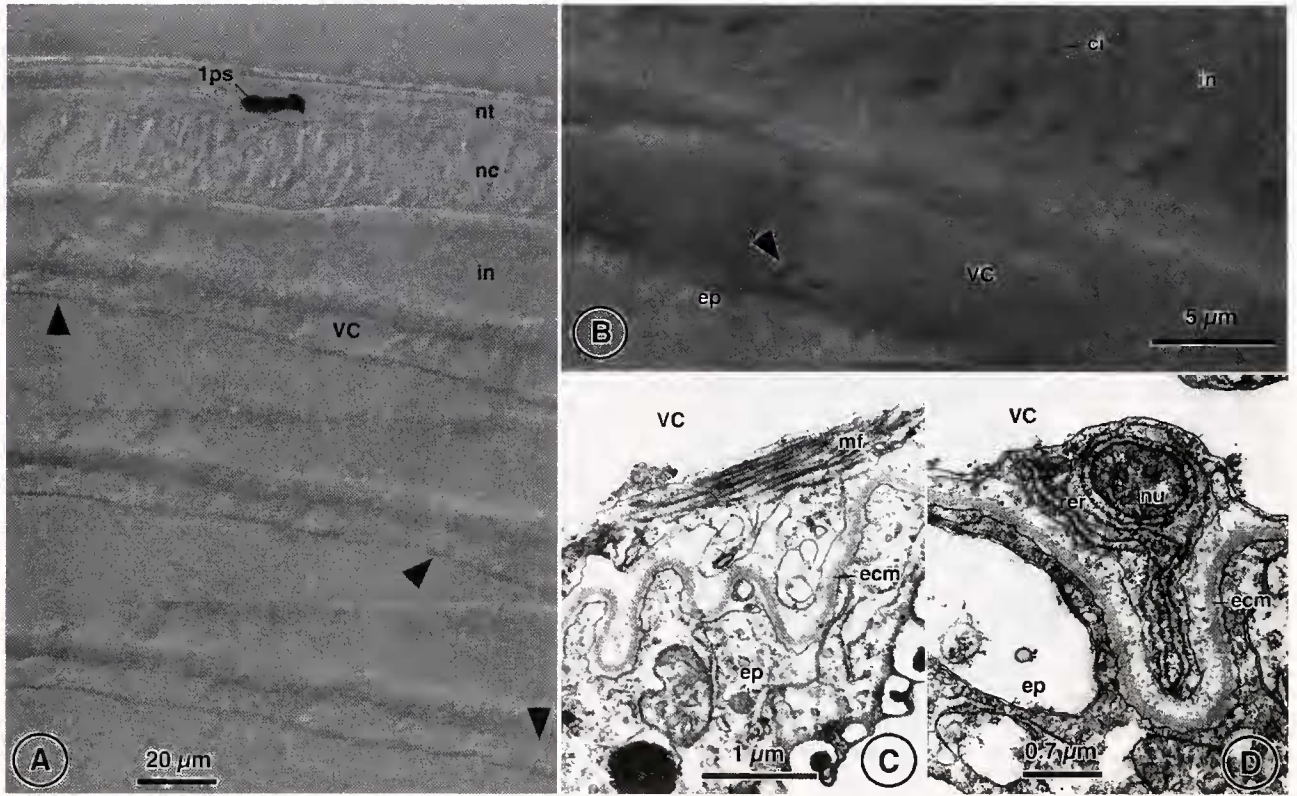


Table 1

Relevant anatomical features and suggested functions of the coelomic system and the blood vascular system of cephalochordates; note: no correlation between anatomical features and functions in the same line intended

Coelom*		Blood vascular system		
	Anatomical features	Functions	Anatomical features	Functions†
Adult	Epithelial lining (with intercellular junctions) present (16) Smooth myoepithelial cells present (16) Free coelomocytes in perivisceral coelom regular (15, 16) Excretory cells (cyrtopodocytes) in nephridia (e.g., 7, 9, 10)	Excretion (e.g., 7, 9, 10) Propulsion of blood (6, 7) Expulsion of gametes (16) Phagocytosis (15) Storage of nutrients (16, 17) Lubrication (16) Hydrostatic skeleton (18)	Part of the blastocoelic space, vessels usually delimited by connective tissue (mostly basement membranes) only: no endothelial lining present (6, 7) Ameboid hemocytes rarely present (e.g., 6, 7, 15) No blood or respiratory pigments present (7)	Endocytosis (19) Ultrafiltration (e.g., 7) ? Nutrient transport ? No respiratory function‡
Larva	Epithelial lining (with intercellular junctions) present (9, 10) Excretory cells (cyrtopodocytes) in Hatschek's nephridium (9, 10) Smooth myoepithelial cells present#	Excretion (9, 10) Distribution of nutrients# ? Secretory function# ? Distribution of secretory products#	Only left anterior aorta distinguishable (10, #) Part of the blastocoelic space, delimited by dense connective tissue <u>not</u> by basement membrane (10, #)	Ultrafiltration (9, 10)

\* Not considering the segmental myocoels, the medial walls of which are specialized as the locomotory trunk muscles.

† Physiology and function of the circulatory system of cephalochordates have hardly been investigated, but the lack of respiratory pigment is generally assumed to indicate that this system does not play a crucial role in oxygen supply.

# Present study.

? Hypothetical function, not fully substantiated.

**Figure 2.** (A) Light microscopic aspect of a peristaltic wave passing along the ventral coelom. Anterior is to the right. About 1.2 s elapsed between first and third frame. Arrowheads, area of contraction (compare to other frames). (B) Higher light microscopical magnification of the ventral coelomic canal. Anterior to the right. Note the particulate matter within the ventral coelom (arrowhead). Direction of movement of the particle was antieriad. (C & D) Transmission electron micrographs of mesodermal cells of a larva (110 h post fertilization, 18°C). (C) Mesothelial cell bearing myofilaments. (D) Cell with extensive profiles of rough endoplasmic reticulum around the nucleus. Such cells may be of secretory function. ci, cilia; ecm, extracellular matrix; ep, ventral epidermis; in, intestine; mf, myofilaments; nc, notochord; nt, neural tube; nu, nucleus; rer, rough endoplasmic reticulum; VC, ventral coelom; Ips, first pigment spot.

### Acknowledgments

I would like to thank Prof. W. Maier for generously providing support and all the facilities of the Lehrstuhl für Spezielle Zoologie, Tübingen. M. Hohloch assisted with the work in the photo lab. Two anonymous reviewers were exceptionally helpful with their constructive critical remarks.

### Literature Cited

- Maisey, J. G. 1986. Heads and tails: a chordate phylogeny. *Cladistics* 2: 201–256.
- Gans, C. 1989. Stages in the origin of vertebrates: analysis by means of scenarios. *Biol. Rev.* 64: 221–268.
- Holland, P. W., L. Z. Holland, N. A. Williams, and N. D. Holland. 1992. An amphioxus homeobox gene: sequence conservation, spatial expression during development and insights into vertebrate evolution. *Development* 116: 653–661.
- Nielsen, C. 1995. *Animal Evolution*. Oxford University Press, New York.
- Rähr, H. 1979. The circulatory system of amphioxus (*Branchiostoma lanceolatum* (Pallas)). A light-microscopic investigation based on intravascular injection technique. *Acta Zool.* 60: 1–18.
- Rähr, H. 1981. The ultrastructure of the blood vessels of *Branchiostoma lanceolatum* (Pallas) (Cephalochordata). *Zoomorphology* 97: 53–74.
- Ruppert, E. E. 1997. Cephalochordata (Acrania). Pp. 349–504 in *Microscopic Anatomy of Invertebrates. Hemichordata, Chaetognatha, and the Invertebrate Chordates 15*. F. W. Harrison and E. E. Ruppert, eds. Wiley-Liss, New York.
- Stokes, M. D., and N. D. Holland. 1995. Ciliary hovering in larval lancelets (= amphioxus). *Biol. Bull.* 188: 231–233.
- Ruppert, E. E. 1996. Morphology of Hatschek's nephridium in larval and juvenile stages of *Branchiostoma virginiae* (Cephalochordata). *Isr. J. Zool.* 42: 161–182.
- Stach, T., and K. Eisler. 1998. The ontogeny of the nephridial system of the larval amphioxus (*Branchiostoma lanceolatum*). *Acta Zool.* 79: 113–118.
- Hatschek, B. 1881. Studien über Entwicklung des *Amphioxus*. *Arbeiten am Zoologischen Institut der Universität Wien und der Zoologischen Station Triest* 4: 1–88.
- Goodrich, E. S. 1934. The early development of the nephridia in amphioxus: introduction and part I. Hatschek's nephridium. *Q. J. Microsc. Sci.* 76: 499–674.
- Drach, P. 1948. Développement de l'amphioxus. Pp. 1001–1027 in *Traité de Zoologie*. P.-P. Grassé, ed. Masson, Saint-Germain, Paris.
- Jefferies, R. P. S. 1986. *Living Acraniates—Amphioxus and Its Relatives*. British Museum (Natural History), London.
- Rhodes, C. P., N. A. Ratcliff, and A. F. Rowley. 1982. Presence of coelomocytes in the primitive chordate amphioxus (*Branchiostoma lanceolatum*). *Science* 217: 263–265.
- Holland, N. D., and L. Z. Holland. 1990. Fine structure of the mesothelia and extracellular materials in the coelomic fluid of the fin boxes, myocoels and sclero-coels of a lancelet *Branchiostoma floridae* (Cephalochordata = Acrania). *Acta Zool.* 71: 225–234.
- Azariah, J. 1965. On the seasonal appearance of fin rays and their bearing on the reproductive cycle of *Branchiostoma lanceolatum*. *J. Mar. Biol. Assoc. India* 7: 459–461.
- Gutmann, W. F. 1971. Was ist urtümlich an *Branchiostoma*? *Nat. Mus.* 101: 340–356.
- Moller, P. C., and C. W. Philpott. 1973. The circulatory system of *Amphioxus* (*Branchiostoma floridae*). II. Uptake of exogenous proteins by endothelial cells. *Z. Zellforsch. Mikrosk. Anat.* 143: 135–141.
- Methods. Developmental stages of *Branchiostoma lanceolatum* were observed alive by means of light microscopy (Leitz, Axioplan) and recorded on videotape (Sony, KSP-60, U-matic). Larvae were fixed for 30 min at 0°C in a glutardialdehyde (8%)–seawater mixture (1:2). Rinsing in seawater was followed by postfixation for 3 h in an OsO<sub>4</sub> (4%)–seawater mixture (1:1). The animals were stained with uranyl acetate en bloc prior to embedding in Epon 812. Serial sections for light and transmission electron microscopy (TEM) were prepared on an LKB ultratome 1 as follows: a survey series of about 20 semi-thin sections 0.5 μm thick was made, followed by a series of ultra-thin sections 0.05 μm in thickness. This pattern was repeated separately for the length and height of the entire animals. Sections were stained with lead citrate (1 min) for TEM and with toluidine blue for light microscopy. TEM micrographs were prepared with a Siemens Elmiskop 102.